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Olfaction in Parkin single and compound heterozygotes in a cohort of young onset Parkinson's disease patients

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Background – Parkin related Parkinson's disease (PD) is differentiated from idiopathic PD by absent or sparse Lewy bodies, and preserved olfaction. The significance of single Parkin mutations in the pathogenesis of PD is debated. Objectives – To assess olfaction results according to Parkin mutation status. To compare the prevalence of Parkin single heterozygous mutations in patients diagnosed with PD to the rate in healthy controls in order to establish whether these single mutations could be a risk factor for developing PD. Methods – Parkin gene mutation testing was performed in young onset PD (diagnosed <50 years old) to identify three groups: Parkin homozygous or compound heterozygote mutation carriers, Parkin single heterozygote mutation carriers, and non-carriers of Parkin mutations. Olfaction was tested using the 40-item British version of the University of Pennsylvania smell identification test (UPSIT). Results – Of 344 young onset PD cases tested, 8 (2.3%) were Parkin compound heterozygotes and 13 (3.8%) were Parkin single heterozygotes. Olfaction results were available in 282 cases (eight compound heterozygotes, nine single heterozygotes, and 265 non-carriers). In Parkin compound heterozygotes, the median UPSIT score was 33, interquartile range (IQR) 28.5–36.5, which was significantly better than in single Parkin heterozygotes (median 19, IQR 18–28) and non-carriers (median score 22, IQR 16–28) (ANOVA $P < 0.001$). These differences persisted after adjusting for age, disease duration, gender, and smoking ($P < 0.001$). There was no significant difference in UPSIT scores between single heterozygotes and non-carriers
($P = 0.90$). Conclusions – Patients with Parkin compound heterozygous mutations have relatively preserved olfaction compared to Parkin single heterozygotes and non-carriers. The prevalence of Parkin single heterozygosity is similar to the 3.7% rate reported in healthy controls.

Introduction

Olfactory dysfunction is an important early pre-motor sign of Parkinson's disease (PD) and is pre-sent in nearly 90% of cases (1). Preserved or mildly impaired olfactory function in a parkinsonian patient is more likely to be related to vascular parkinsonism or atypical parkinsonism such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), or corticobasal degeneration (CBD), whereas markedly reduced olfaction is more suggestive of idiopathic PD (2, 3).

Bi-allelic Parkin mutations causing parkinsonism have been postulated as a different diseaseentity compared to idiopathic PD, based on young age at onset (invariably before age 50 years), relatively preserved olfactory function (4), and absent or sparse Lewy bodies (5).

Impaired olfaction may predate a clinical diagnosis of PD by at least 4 years, based on population data from older males, and is proposed therefore as a useful screening tool for 'at risk' subjects (e.g., those with a positive family history) to detect those at a higher risk for later development of PD (6). Parkin mutations are inherited in an autosomal recessive manner; Parkin mutations can be single heterozygous (i.e. a mutation in 1 allele), homozygous (i.e. the same mutation in 2 alleles), or compound heterozygous (i.e. different mutations in 2 alleles). Young age at onset, race/ethnicity, and family history of PD are predictors of carrying Parkin mutations (7).

Earlier studies indicated that olfaction is not impaired among patients with PD who have Par-kin compound heterozygote mutations (or homozygote status). These patients had significantly higher University of Pennsylvania smell identification test (UPSIT) scores compared to single heterozygotes or non-carriers, and the differences persisted after adjustment for other factors that influence olfactory scores, namely age, gender, and disease duration (8). This then raises the question whether single Parkin mutations are incidental to the diagnosis of PD, rather than playing a role in the development of the PD phenotype when present (9). The background prevalence of Parkin single heterozygosity is 3.7% in healthy subjects, which is a useful reference figure for comparison to a PD cohort (10).

We systematically evaluated olfactory function and Parkin mutation status in a cohort of young onset PD cases to define the prevalence and severity of olfactory dysfunction in Parkin mutation carriers with PD classified by genotype (single heterozygous, compound heterozygous or homozygous) and non-carriers.

Methods

Subjects

Participants in the primary analysis were enrolled in the Tracking Parkinson's study, a multicentre study in the United Kingdom (UK) which includes two PD patient cohorts: young onset cases (diagnosed at age <50 years), and recent onset cases (diagnosed within the last 3 years). The study protocol includes olfactory assessment and detailed motor, non-motor, and quality-of-life scoring. The diagnosis of PD was made according to the Queen Square Brain Bank criteria (11.) Cases who were diagnosed with vascular, drug-induced, and atypical parkinsonism (MSA, CBD, PSP) were excluded (12). Parkin mutation status was

determined, for practical reasons, in patients diagnosed under 50 years of age in the young onset cohort, and under 45 years of age in the recent onset cohort, making a total of 344 cases. Cases were classified into three groups: bi-allelic Parkin mutation carriers, single Parkin mutation carriers, and Parkin non-carriers. The study procedures were approved by the West of Scotland Research Ethics committee and performed according to the Declaration of Helsinki. All subjects provided written informed consent.

Olfaction

Olfaction testing was performed using the British version of the 40-item UPSIT test (13). These smell tests were self-administered in outpatient clinics; patients were given verbal guidance which followed standard procedures for this test, requesting an obligatory choice from one of four options for each odor.

Where one question was unanswered, the total of the 39 answered questions was scaled up by multiplying the total by 40/39. If <39 questions were answered, then the olfaction scores were not used.

Where one question was unanswered, the total of the 39 answered questions was scaled up by multiplying the total by 40/39. If <39 questions were answered, then the olfaction scores were not used.

The centile position for the olfaction score, corrected for age and sex, was estimated from normative data charts provided by the manufacturer (14), after smoothing the data curves using LOWESS techniques, and results at or below the 15th centile were considered as hyposmic.

Patients with temporary nasal obstruction from viral or similar causes had their assessment deferred, and patients with sinunasal disorders were not tested.

Genetic testing

Whole-genome DNA amplification was per-formed and then used for mutational screening by direct sequencing in Parkin, PINK1 and GBA. LRRK2 genotyping for the G2019S point mutation using a ‘Kompetitive’ allele-specific polymerase chain reaction (KASP) assay (LGC Genomic solutions) was also performed. Copy number variation in the Parkin gene was analyzed by multiplex ligation-dependent probe amplification (MLPA) using specific kits (obtained from MRC Holland) according to the manufacturer’s protocol.

Additional assessments

While the primary objective was the examination of olfaction in relation to Parkin mutation status, detailed motor and cognitive assessments were also performed. Motor function was assessed according to part 3 of the Movement Disorder Society unified PD rating scale (MDS-UPDRS3) and Hoehn and Yahr staging (15, 16). Cognition was assessed using the Montreal cognitive assessment (MoCA) questionnaire (17). The non-motor symptom score (NMSS) was calculated using the validated 30-item NMSS scale (18). Levodopa equivalent daily dose was calculated by an established dose equivalence method (19).

Statistical Analysis

University of Pennsylvania smell identification test scores were calculated as the number of correct answers provided, ranging from 0 to 40. Statistical analysis was performed with the UPSIT scores as the primary outcome, correcting for age, gender, and smoking status (categorized into never, passive, ex, or current smoker) as olfactory scores are known to decline with increasing age, and are lower in males and smokers.
Fisher's exact test for categorical variables and ANOVA (where the residuals were normally disributed) or Kruskal–Wallis (where residuals were not normally distributed) tests for continuous variables were performed for univariate comparisons among groups for demographic features. For the primary outcome of UPSIT olfactory performance, multivariable linear regression models were used to compare UPSIT scores for the three patient groups (Parkin compound heterozygotes, Parkin single heterozygotes, and non-carriers of the Parkin mutation), adjusting for age (as a continuous variable), gender, disease duration (as a continuous variable), and smoking behavior as defined above. We checked the linearity assumption for age and disease duration by analysing univariate models, with the variables grouped into quintiles. Residuals from the UPSIT regression models were examined for normality. We then derived the mean differences in UPSIT scores for compound and single heterozygotes compared to non-carriers conditioned on being male, non-smoking, and of average age and dis-ease duration.

Results

Of 344 young onset PD cases, 8 (2.3%) were Par-kin compound heterozygotes and 13 (3.8%) were Parkin single heterozygotes. There were 9 (2.6%) LRRK2 mutation-positive cases, 44 (12.8%) GBA cases, and 10 (2.9%) PINK1 cases. Demographics and univariate comparisons are described in Table 1. Olfaction, motor, and non-motor scores are shown in Table 2. Olfaction results were inadequate (<39 odors scored) in 62 cases, leaving 282 cases for analysis. There were no significant age, sex, cognition, or motor score differences between patients completing the olfaction test successfully, compared to those in whom test results were incomplete (data not shown). Of the 282 scores that were assessed, 276 had scored all 40 odor scores (97.9% of cases with usable olfaction results) and six had scored 39 of 40 odor scores (2.1% of cases with usable olfaction results). In the Parkin mutation-positive cases, usable olfaction results were available in 17 of 21 cases (eight of eight compound heterozygotes, and nine of 13 single heterozygotes).

Table 1 Demographic characteristics in 344 patients with young onset PD tested for Parkin mutations

	Compound heterozygotes $n = 8$	Single heterozygotes $n = 13$	Non-carriers $n = 323$	*P*-value
Current age (years)	52.7 (44–59)	47.3 (44–59)	50.9 (47–56)	0.96[a]
Age at diagnosis (years)	37.5 (27–43)	40.5 (38–45)	44.8 (41–48)	0.001[b]
Disease duration (years)	17.1 (7–24)	9.7 (4–20)	5.5 (2–11)	0.008[b]
Gender (male, %)	4 (50%)	7 (53.9%)	214 (66.3%)	0.41[c]
Handedness (right, %)	7 (87.5%)	13 (100%)	273 (85.3%)	0.64[c]
Family history of PD (%)	4 (50.0)	3 (23.1%)	82 (25.4%)	0.34[c]
Smoking (%)				
Never	2 (25.0%)	4 (36.4%)	93 (34.1%)	0.21[c]
Passive	3 (37.5%)	7 (63.6%)	85 (31.1%)	
Previous	3 (37.5%)	0	72 (26.4%)	
Current	0	0	23 (8.4%)	

Numbers are median (interquartile range) except where indicated.
[a]ANOVA (residuals were normally distributed).
[b]Kruskal–Wallis (residuals not normally distributed).
[c]Fisher's exact.

Table 2 Olfactory, motor, non-motor, and cognitive scores in young onset PD cases tested for Parkin mutations

	Compound heterozygotes $n = 8$	Single heterozygotes $n = 13$	Non-carriers $n = 323$	*P*-value
UPSIT score[a]	33 (28.5–36.5)	19 (18–28)	22 (16–28)	<0.001[b]
Hyposmic[a](%)	2 (25.0%)	7 (77.8%)	238 (89.8%)	<0.001[c]
MDS-UPDRS 3 score	29 (12–60)	32 (16–44)	22 (13–34)	0.34[d]
Hoehn and Yahr stage	2.75 (2–3.5)	2.5 (1–2.5)	2 (1.5–2.5)	0.05[d]
MoCA	27.5 (26.5–29)	25 (24–26)	26 (24–28)	0.08[d]
NMSS	39 (18–53)	41 (22–78)	41 (23–74)	0.95[d]
LEDD (mg/day)	400 (160–1100)	960 (500–1425)	640 (325–1040)	0.19[d]

Numbers are median (interquartile range) except where indicated.
In Parkin compound heterozygotes, the median UPSIT score was 33, inter-quartile range (IQR) 28.5–36.5, which was significantly better than in Parkin single heterozygotes (median 19, IQR 18–28) and non-carriers with a diagnosis of PD (median score 22, IQR 16–28) (ANOVA $P < 0.001$). These differences persisted after adjusting for age, disease duration, gender, and smoking behavior ($P < 0.001$). Although UPSIT generates an ordinal score, all residuals were normally distributed. There was no significant difference in olfaction scores between Parkin single heterozygotes and non-carriers ($P = 0.90$), which persisted after adjustment for covariates.
Using our multivariable model, the average UPSIT score for a Parkin mutation non-carrier was 19.1 for a male, non-smoker with an average age of 52.3 years, and average disease duration of 8.5 years. In comparison with non-carriers, the change in UPSIT score for a single

heterozygote with the same characteristics was 0.09 (95% CI: 4.4 to 4.3; P-value = 0.97) and for a compound heterozygote was 11.5 (95% CI: 6.8–16.3; P-value <0.001).
Results from statistical comparisons between groups in Tables 1 and 2 were similar after excluding cases which tested positive for LRRK2, GBA, or PINK1 (data not shown).

Discussion

The prevalence of Parkin mutations in young onset PD cases varies by ethnicity, and country of origin, with reported prevalence ranges ranging from 2.9% to 11.1% (20–23). The 2.3% prevalence rate of Parkin-related disease (compound heterozygote or homozygote mutations) in the present study is comparable to a previous systematic review of young onset PD patients from the UK (24). The 3.8% rate of Parkin single heterozygotes in our study is almost identical to that in a US study (3.9% of 956 young onset PD cases)(7), and to the background rate of 3.7% in healthy subjects (10).
While autosomal recessive young onset parkinsonism caused by Parkin gene mutations was Discussion initially described in Japan as one of the mono-genic forms of PD, a wide variety of mutations and (less commonly) deletions in the Parkin gene can cause parkinsonism in people of European ancestry (25). None of our patients had homozygous Parkin mutations, which is again consistent with prior UK observations, while noting that other European studies found homozygous Par-kin mutations to be more common than heterozygous Parkin mutations (24, 26).
We applied the British version of the UPSIT test, developed by Sensonics Inc (Haddon Heights, NJ, USA), which is largely similar in design and use to their original United States (US) version. However, the British version replaces a limited number of odors and corresponding choices (e.g. root beer) with more familiar odors for UK users. As the UK and US versions of the UPSIT retain sufficient homology in administration and interpretation of data, it is reasonable to compare olfaction score results across studies using these analogous versions. This approach appears preferable to the application of the US version to UK subjects, which risks misclassification of normal individuals as mildly hyposmic, unless weighted scores are calculated after omitting the less familiar odors (27).
Olfaction has been the subject of few prior stud-ies with detailed Parkin mutation analysis in patients with PD. Olfaction in Parkin-related dis-ease is reported to be normal, or near normal, and differs significantly from patients with young onset PD without Parkin mutations and from cases diagnosed as ‘idiopathic’ PD. This along with neuropathologic and clinical characteristics such as long disease duration has led some to hypothesize that young onset PD due to Parkin gene mutations is a separate disease entity (4). More recently, a large US study showed that olfaction in young onset PD is related to Parkin gene mutation status, and those with compound heterozygote mutations in this gene have preserved olfactory function, unlike those with single heterozygote mutations and non-carriers. Our results are largely similar to and validate the observations from the US study (7, 8). The differences in UPSIT scores in both studies remained significant even after adjustment for age, gender, disease duration, and smoking status. Our observations therefore lend support to the hypothesis that olfactory loss in cases of PD with Parkin single heterozygosity may be due to their having ‘idiopathic’ PD and that the Parkin single mutation status may be

incidental, as opposed to the well-recognized phenomenon of preserved olfaction in Parkin-related disease (compound heterozygotes and homozygotes). However, the possibility that Parkin single heterozygosity could coexist with other risk factors and lead to PD can-not be excluded by our olfaction results. It is also interesting to note that preserved olfaction in Par-kin compound heterozygotes so far appears unique among the monogenic types of PD, where olfactory loss generally matches that of 'idiopathic' cases of PD (28–30), although one study found less marked olfactory loss in PD cases with LRRK2 mutations than those with sporadic PD (31).

While we observed group differences in UPSIT scores that were statistically significant, when comparing Parkin compound heterozygotes to Parkin single heterozygous mutation carriers and non-carriers with PD, caution is required when interpreting the results of olfaction testing in the individual patient. Two of our eight Parkin com-pound heterozygotes, with PD durations of 18 and 29 years, were hyposmic scoring below the 15th centile using UPSIT normative data. Further, some of our Parkin single heterozygotes, and some of our non-carriers were normosmic. Accordingly, olfactory test results alone cannot be used to predict Parkin mutation status.

The preserved olfaction in Parkin compound heterozygotes (and presumably homozygotes) compared to 'idiopathic' PD is one component of a wider pattern of distinguishing features. Young age at onset, more symmetric involvement, dystonia at presentation, brisk deep tendon reflexes, a good response to levodopa therapy (32), and more symmetric and more marked reduction of dopamine uptake on 123I FP-CIT SPECT scan (DaTSCAN) are found in Parkin-related disease (33, 34). Parkin mutation carriers (homozygous or compound heterozygous) also show better cognitive and motor performance than non-carriers, suggesting slower disease progression in cross-sectional studies, although longitudinal studies are required to confirm this (35). Parkin bi-allelic mutation carriers have less extensive distribution of Lewy bodies neuropathologically, that may not follow Braak staging, and spares olfactory structures (5). The sparing of olfactory structures in Parkin-related disease may explain their pre-served olfaction.

There are some limitations to our study such as a lack of an internal control group of healthy people. This meant that we had to compare the rate of Parkin single heterozygosity in our study cohort to that published in the literature for nor-mal healthy subjects, and while we have tried to answer the question about the relevance of Par-kin single mutations in young onset PD, this study has not answered the question of their relevance to PD in general. We are now undertaking a more detailed analysis of the olfactory testing in our larger cohort of recent onset PD cases, to examine for factors that associate with preserved olfaction, as well as features that correlate with moderate to severe hyposmia in the early stages of disease.

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Conflicts of interest

N Malek, DMA Swallow, KA Grosset, MA Lawton, Y Ben-Shlomo, C Bresner, N Williams, and N Wood declare no conflicts of interest.

N Bajaj has received payment for advisory board atten-dance from UCB, Teva Lundbeck, Britannia, GSK, Boehringer, and honoraria from UCB Pharma, GE Healthcare, Lily Pharma, Medtronic. He has received research grant support from GE Healthcare.

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